Mass Cytometry Reveals Distinct Platelet Subtypes in Healthy Subjects and Novel Alterations in Surface Glycoproteins in Glanzmann Thrombasthenia

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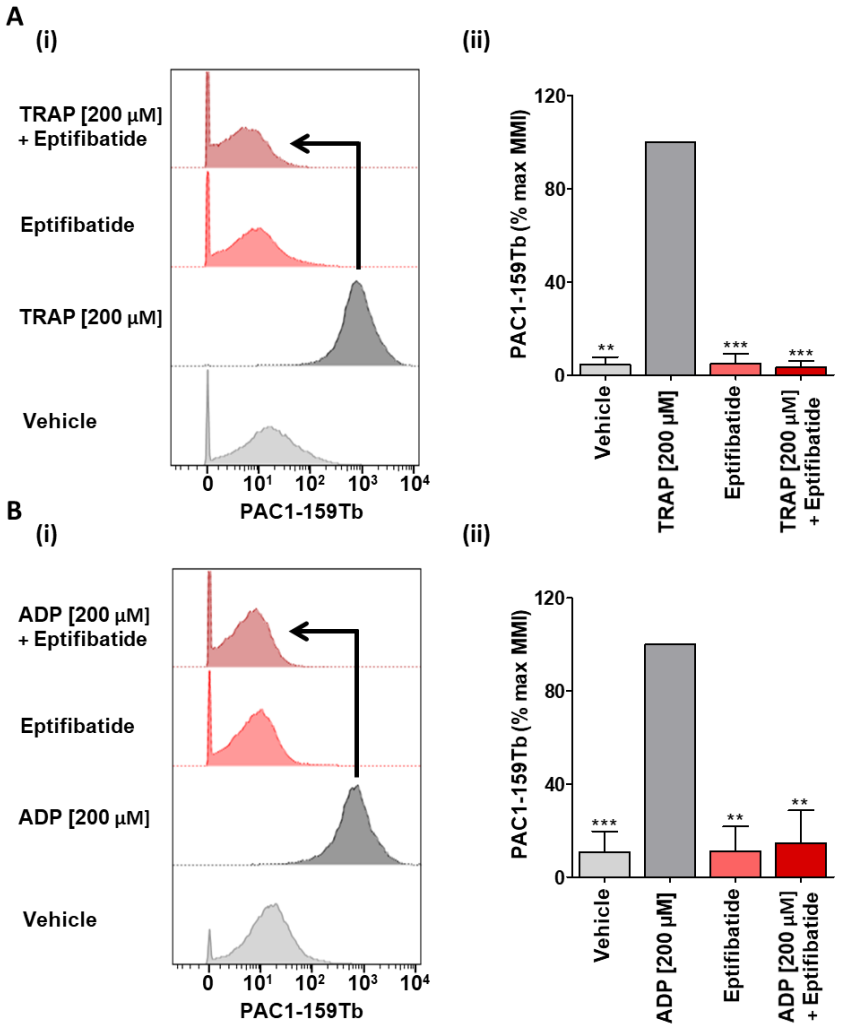
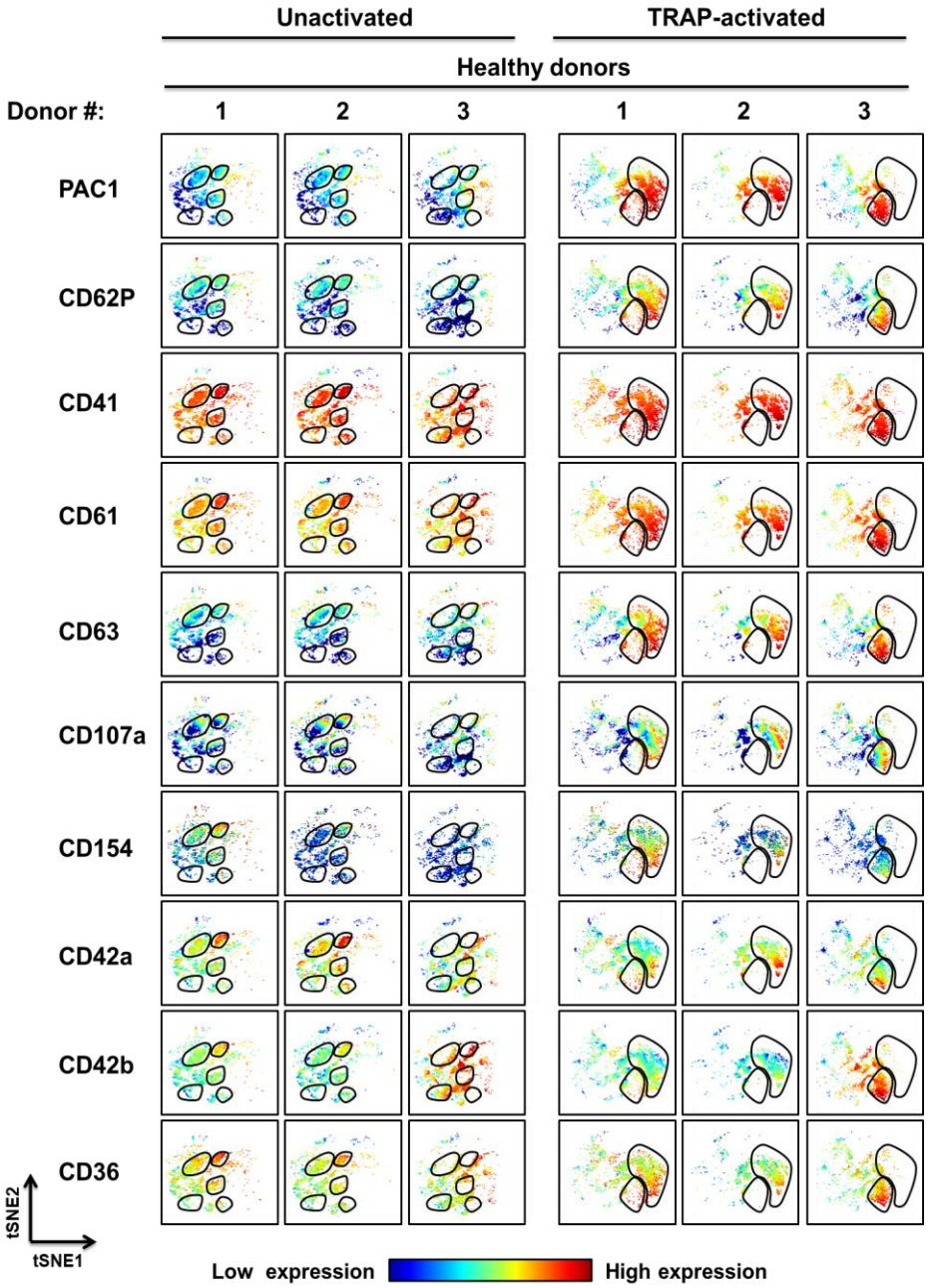
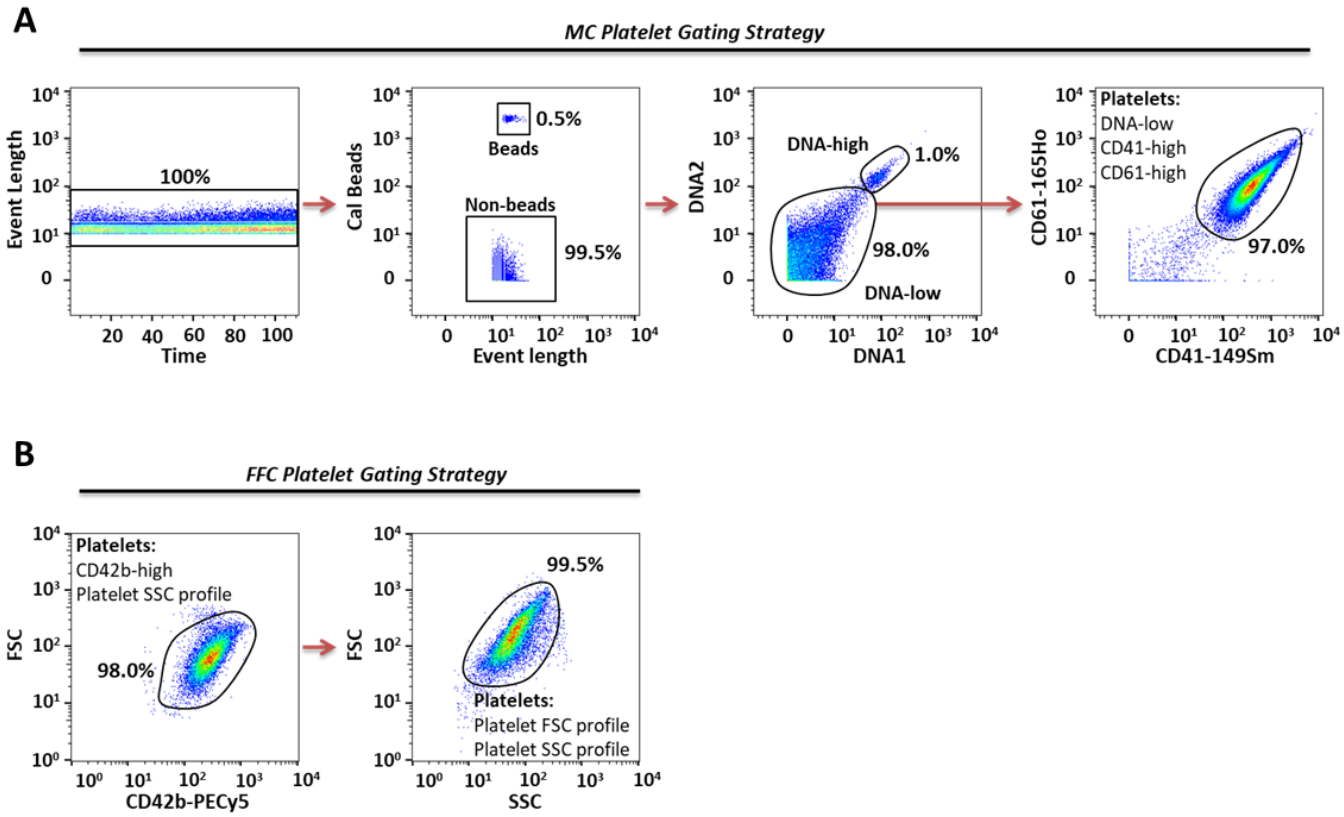


Supplemental Figure S1. Measuring the specificity of in-house metal-tagged PAC1 for integrin αIIbβ3. (A-B) Citrate-anticoagulated blood was treated with 200 µM TRAP/ADP, 3.33 µg/mL eptifibatide or TRAP/ADP plus eptifibatide in combination for 30 minutes in the presence of PAC-1-159Tb. Samples were fixed in 1% formaldehyde and analyzed by mass cytometry. Representative histograms demonstrating the mean metal intensity (MMI) are displayed **(A(i), B(i))** along with bar charts with results expressed as a percentage of the MMI achieved with 200 µM TRAP/ADP (mean ± SEM; n=3 **(A(ii), B(ii))**). Statistical analysis: 1-way ANOVA was used in conjunction with a Bonferroni post-test (with all results compared to the MMI achieved with agonist stimulation) to indicate statistical significance; **P<0.01 and ***P<0.001. Abbreviations: ADP, adenosine diphosphate; TRAP, thrombin receptor activating peptide.



Supplemental Figure S2. Multidimensional analysis of platelets by MC reveals common and private platelet subpopulations in 3 different healthy donor samples. Visual stochastic neighbor embedding (viSNE) plots of whole blood samples drawn from 3 separate healthy donors. Samples were stained with a metal-tagged antibody cocktail containing 10 markers (directed against: CD36, CD41, CD42a, CD42b CD61, CD63, CD62P, CD107a, CD154 and PAC1), treated with vehicle of 20 µM TRAP, and analyzed using MC. Color intensity relates to antigen expression (low [blue] or high [red]) and each dot represents an individual platelet. The distance between dots/platelets and populations of dots/platelets is inversely proportional to how closely related those dots/platelets are in terms of antigen expression and characteristics. Abbreviations: TRAP, thrombin receptor activating peptide; tSNE, t-distributed stochastic neighbor embedding.



Supplemental Figure S3. Platelet gating strategy for MC and FFC. Platelets are identified as DNA-low and CD41/CD61-high by MC **(A)**. For Glanzmann thrombasthenia studies platelets are identified as DNA-low and CD42a/CD42b-high by MC. Platelets are identified by typical forward- and side-scatter properties and CD42b-high by FFC **(B)**. Abbreviations: DNA, deoxyribonucleic acid; FFC, fluorescence flow cytometry; FSC, forward-scatter MC, mass cytometry; SSC, side-scatter.

Antigen	Common name	Clone	Antibody type	Metal tag	Tag type	Manufacturer	Final conc. (μg/mL)
CD9	Tetraspanin	SN4 C33A2	IgG; monoclonal	171Yb	C	Fluidigm	5
CD29	Integrin β1	TS2/16	IgG; monoclonal	176Yb	I	Biolegend	5
CD31	PECAM-1	WM59	IgG; monoclonal	145Nd	C	Fluidigm	5
CD36	GPIV	5-271	IgG; monoclonal	150Nd	C	LMAAC	2
CD42a	GPIX	ALMA.16	IgG; monoclonal	155Gd	I	BD Biosciences	2.5
CD42b	GPIbα	HIP1	IgG; monoclonal	163Dy	C	LMAAC	3.5
CD41	Integrin αIIb	HIP8	IgG; monoclonal	149Sm	C	LMAAC	2
CD62P	P-selectin	AK4	IgG; monoclonal	172Yb	C	LMAAC	3.5
CD61	Integrin β3	VI-PL2	IgG; monoclonal	165Ho	C	Fluidigm	5
CD63	LAMP-3	H5C6	IgG; monoclonal	161Dy	C	LMAAC	3.5
CD107a	LAMP-1	H4A3	IgG; monoclonal	166Er	C	LMAAC	3.5
CD154	CD40L	24-31	IgG; monoclonal	154Sm	C	LMAAC	3.5
GPVI	GPVI	N/A	IgG; polyclonal	152Sm	I	EMD Millipore	7.5
Activated αIIbβ3	Activated αIIbβ3	PAC-1	IgM, monoclonal	159Tb	I	BD Biosciences	7.5-17

Supplemental Table S1. A list of metal-tagged antibodies used for MC experiments. Abbreviations: C, commercial; CD, cluster of differentiation; GP, glycoprotein; I, in-house; Ig, immunoglobulin; LMAAC, Longwood Medical Area Antibody Core; MC, mass cytometry.

Antigen	Common name	Clone	Antibody type	Fluorescent tag	Tag type	Manufacturer	Final conc. (μg/mL)
CD41a	Integrin αIIb	HIP8	IgG, monoclonal	PE	C	BD Biosciences	1:15 final dilution of stock
CD42b	GPIbα	HIP1	IgG, monoclonal	PE-Cy5	C	BD Biosciences	1
CD61	Integrin β3	Y2/51	IgG, monoclonal	FITC	C	Agilent	1:125 final dilution of stock
CD62P	P-selectin	AK4	IgG, monoclonal	PE	C	BD Biosciences	1.5
Activated αIIbβ3	Activated αIIbβ3	PAC1	IgM, monoclonal	FITC	C	BD Biosciences	40

Supplemental Table S2. A list of fluorescent-tagged antibodies used for FFC experiments.
Abbreviations: C, commercial; CD, cluster of differentiation; Cy, cyanine; FFC, fluorescence flow cytometry; FITC, fluorescein isothiocyanate; GP, glycoprotein; Ig, immunoglobulin; PE, phycoerythrin.